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Captured Diversity in a Culture Collection: Case Study of the Geographic and Habitat Distributions of Environmental Isolates Held at the American Type Culture Collection

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The use of molecular techniques to assess prokaryotic diversity independent of the need for enrichment culture has profoundly changed how we view and study microbial diversity. As molecular data have accumulated over the past 15 years, these data are now resulting in a healthy debate about how much we really know about prokaryotic diversity in a wide range of environments (8, 21–23, 32, 44, 55). A number of recent articles have discussed these questions, and opinions range from the position that prokaryotic diversity is essentially infinite, with the existence of millions of potential species, (6, 10), to arguments that microbial diversity is reasonably finite (17, 26).

In light of this debate concerning the results of theoretical considerations and molecule-based surveys, it is interesting to take stock of the holdings of the environmental prokaryotes available at the American Type Culture Collection (ATCC) to determine how these holdings reflect overall trends in microbial diversity in different habitats. The principle mission of bioresource centers, such as the ATCC, is to serve as living stock collections that acquire diverse biological materials for redistribution to researchers throughout the world. The ATCC has about two-thirds of the type strains of prokaryotic species in the world. In many cases, multiple strains of a given species are acquired. As these materials are accessioned, data relevant to their provenance and physiology are collected. These data are an underutilized asset of bioresource centers. For example, the data on the source locations of isolates can provide information about the relative sampling efforts for different geographic regions. Concordantly, data concerning the environmental habitats of isolates can provide important information about the diversity of genera that are associated with certain habitat types. No articles were found in a literature search that specifically analyzed culture collections as a metric for assessing our knowledge of larger patterns of microbial diversity.

In this article we present an analysis of the holdings of environmental prokaryotes at the ATCC with regard to the geographical and environmental habitats from which they were isolated. The term environmental is broadly defined to include all organisms that are not pathogenic to humans or animals or that are not otherwise human associated (see below). Selected habitats for which holdings at the ATCC are the most abundant were compared to recently published findings of workers who used cultivation-independent methods to assess microbial diversity. This provided perspective on how well the "captured diversity" in a culture collection represents the diversity which may exist in nature.

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ANALYSIS OF THE ATCC ENVIRONMENTAL HOLDINGS

Organisms included in this study were chosen from the approximately 18,000 accessions in ATCC's prokaryotic database. The focus was environmental; therefore, any sample obtained from a human donor or presumed to be associated with a human was not included. If a body part was listed without reference to the animal from which it came (for example, perineal abscess), it was assumed to be human and not included. Human or animal pathogens were not included. Any accession that was not catalogued or was under patent protection was not considered. Additionally, organisms that were described as mutations or laboratory contaminants were not considered. Thirty-nine broad categories of environmental habitat were delimited (Table 1). Allocations were determined by isolation data from ATCC's databases. The result was a total of 5,341 strains that met these criteria and could be assigned to a habitat. Some accessions did not fit neatly into one category; if, for example, an organism was isolated from a microbial mat in a marine hot spring, that would allow its assignment to at least two environmental habitats. While such duplications would result in an organism being tallied twice for different habitats, from a survey of the data it was calculated that less than 1% of the total number were duplicates.

Geographically, the world was divided into the six inhabited continents plus the polar regions. The accessions from the polar regions comprised those from Antarctica, as well as any accessions described as derived from the Arctic Circle for which no further geographic information was available. Pacific island nations were included in Oceania, along with Australia and New Zealand. The western Russian border served as the line of demarcation between Europe and Asia; as the majority of Russia is on the Asian continent, Russia was listed with the Asian nations. The North America category included all of the countries in Central America. Isolates from the open ocean (i.e., away from the continental margins) were assigned to the closest continental landmass. While this was somewhat arbitrary, there were so few of these accessions that their numbers had little impact on the overall geographic distribution.

Historical isolation data were listed under the nation's current name whenever possible; for example, organisms isolated

TABLE 1. Distribution of accessions by environmental habitat

Code	Explanation and assumptions	No. of accessions	% of total
SOI	Soil	1,459	27.3
HTP	Host associated, terrestrial, plant	852	16.0
FPR	Food, food processing	388	7.3
HTM	Host associated, terrestrial, mammal	286	5.4
SWM	Sewage or manure	262	4.9
MAC	Marine, coastal	232	4.3
FRL	Freshwater, lake	201	3.8
IND	Industrial processes or waste	153	2.9
HST	Hot springs, terrestrial	152	2.8
HMA	Host associated, marine, animal	139	2.6
HTI	Host associated, terrestrial, insect	132	2.5
ASM	Aqueous sediment, marine	114	2.1
SAL	Saltern, salt lake, or salt flat	109	2.0
ASF	Aqueous sediment, freshwater	108	2.0
WAT	Water, nondrinking	91	1.7
FRR	Freshwater, river	69	1.3
HFF	Host associated, freshwater, fish	66	1.2
COM	Compost, including silage and all composted material	51	1.0
HTA	Host associated, terrestrial, animal	50	0.9
HTB	Host associated, terrestrial, bird	49	0.9
EST	Estuary or estuarine sediment	46	0.9
HSM	Hot springs, marine	44	0.8
HFP	Host associated, freshwater, plant	35	0.7
HMP	Host associated, marine, plant	34	0.6
MMB	Microbial mat or biofilm	31	0.6
SOIc	Soil, contaminated	31	0.6
HAF	Host associated, fungus	30	0.6
AIR	Air, airborne	27	0.5
UNK	Unknown	25	0.5
MMM	Man-made materials	22	0.4
ASMc	Aqueous sediment, marine, contaminated	17	0.3
ASFc	Aqueous sediment, freshwater, contaminated	14	0.3
MOO	Marine, open ocean	8	0.1
FRLc	Freshwater, lake, contaminated	5	0.1
FRRc	Freshwater, river, contaminated	5	0.1
ESTc	Estuary or estuarine sediment, contaminated	3	0.1
MACc	Marine, coastal, contaminated	1	0.02

in Rhodesia were assigned to Zimbabwe, but organisms isolated in "the East Indies" could not be placed in a contemporary nation-state with any confidence. If USSR was the sole geographic designation without any further information, the accession was assigned to Russia. If the United Kingdom or Great Britain was the sole geographic designation, the accession was assigned to England. The Czech Republic and Slovakia were listed jointly, as most accessions did not give additional information (a city name or geographical landmark) that could be used to assign it to one country or the other. Finally, isolation data listed only as Yugoslavia were assigned to the rump Yugoslavia if no city was mentioned.

Organisms were designated by ATCC numbers in a Microsoft Excel spreadsheet with the environmental habitats as columns and geographical locations as rows. The ATCC numbers were later translated into names and grouped by genus.

DATA ASSESSMENT

Begun in 1925, the ATCC is the oldest continuously operating culture collection in the world. One of the goals of the Bacteriology Collection is to maintain as comprehensive a collection of the type strains of prokaryotes as possible. Solicitations for newly described species are made to scientists all over the world, and culture exchanges have been carried out with several other national collections in Europe and Asia. Nevertheless, the holdings at the ATCC will always be somewhat biased by its location in the United States. It is legitimate to ask whether the ATCC reflects global trends in the diversity of its isolates, or if its holdings are overly influenced by its location in North America to be of more general use. Two facts suggest that the ATCC does represent a reasonable benchmark for assessing overall microbial diversity held in culture collections. In terms of environmental isolates that could be assigned to a geographic location, 65% came from outside the United States. While the largest fraction of deposits is from the United States, the distribution of isolates is worldwide (Table 2). The second fact in support of the conclusion that the ATCC is a truly cosmopolitan collection is that two-thirds of the 6,607 type strains of validated species are held in the ATCC's Bacteriology Collection.

Another issue having to do with the usefulness of the data is the fact that only about 60% of the strains at the ATCC that fit our criteria of "environmental isolates" could be assigned to a geographic location (country and/or state). A check of the ATCC's internal records suggested that in >95% of the 40% remaining or unknown cases this information was not available. In the current study we did not examine the original

TABLE 2. Distribution of accessions by continent

Continent	No. of countries	No. of entries	% of total entries
Unknown	NA[a]	2,209	41.4
North America	13	1,289	24.1
Europe	28	784	14.7
Asia	33	612	11.5
Africa	26	149	2.8
Oceania	11	135	2.5
South America	13	94	1.8
Polar regions	NA	69	1.3

[a] NA, not applicable.

citations describing a deposit to determine the extent to which geographic information was never recorded for a given strain. By contrast, at the ATCC more emphasis has been placed recently on recording a strain's geographic origin. This should be a goal of all taxonomists and culture collections, since this information is of great utility in assessing global trends in capturing microbial biodiversity.

GEOGRAPHIC DATA

Table 2 gives the major geographic breakdown of isolates listed by continent. Not surprisingly, North America and Europe accounted for the majority of deposited strains. Nevertheless, a total of 124 nations (including 65% of the current 191 member states of the United Nations) were represented. The three countries with the largest numbers of accessions were the United States, Japan, and Germany, with 1,135, 300, and 189 accessions, respectively.

As the ATCC is located in the United States, it is not surprising that entries from the United States comprised 88% of the North American accessions and 36% of all accessions that could be assigned to a geographical location. Within the United States, California had the most entries with 201, which accounted for 17.7% of all American entries. A full 50% of the entries from California were evenly split between the host-associated terrestrial plant (HTP) and soil (SOI) habitats (Fig. 1). Given that California is the most populous American state and has significant and diversified agricultural interests and a large state university system, it is not surprising that it leads the United States in accessions. More surprising is that Hawai'i and Wyoming (which rank 42nd and 50th in population, respectively) are ranked two and three in terms of deposits. Hawai'i had 87 entries, or 7.7% of the United States entries, 75% of which were from the coastal marine (MAC) habitat. Wyoming had 53 accessions or 4.7% of the total from the United States. Hawai'i appears to have a few individuals who have worked hard at isolating and describing a select group of marine isolates. Most of the isolates from Wyoming originated from Yellowstone National Park; this reflects the growing interest among microbiologists in extremophiles and shows how well-managed, remarkable geologic features, in this case the various hot springs, can influence the collection of isolates. The remainder of the accessions from the United States (70%) were evenly distributed among the remaining states, with a general trend toward more accessions from the more populous states.

For Europe, Germany, France, and England were the three leading sources for deposits, accounting for 24%, 12.4%, and 10% of the accessions, respectively. This follows from the fact these three countries have the greatest scientific output of the European countries. Germany, especially, has a rich history of studying organismal microbiology. Similarly, Japan was the source of 49% of all the isolates from Asia. India (13%),

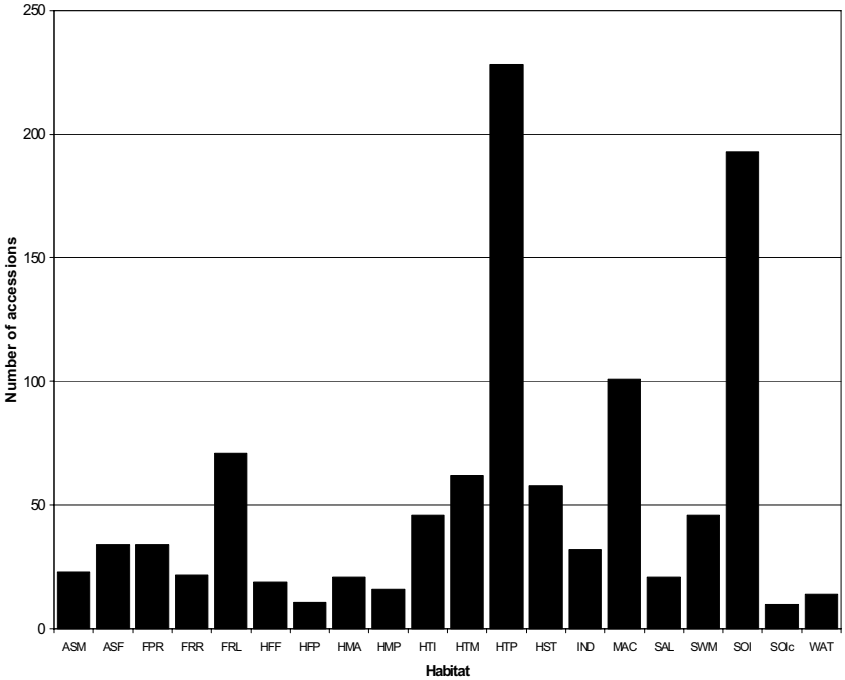

FIG. 1. United States environmental accessions by habitat. See Table 1 for an explanation of the codes.

TABLE 3. Comparison of numbers of known species in biodiversity hotspots[a]

Continent	Biodiversity hotspot	No. of plant species	No. of terrestrial vertebrate species	No. of microbial species
South America	Tropical Andes	45,000	3,389	31
North America	Mesoamerica	24,000	2,859	55
Asia-Pacific	Sundaland	25,000	1,800	34
Europe and Central Asia	Mediterranean Basin	25,000	770	314
South America	Atlantic Forest	20,000	1,668	48
Mainland Asia	Indo-Burma	13,500	2,185	138
North America	Caribbean	12,000	1,518	41
Mainland Asia	Mountains of southwest China	12,000	1,141	121
Africa	Madagascar and Indian Ocean islands	12,000	987	5
South America	Brazilian Cerrado	10,000	1,268	38

[a] For geographical definitions of biodiversity hotspots, see www.biodiversityhotspots.org/xp/Hotspots. Many of these hotspots do not conform to geographical boundaries. Portions of countries may be included, or, alternately, different locations in one country may belong to two or more hotspots. For the purposes of this comparison, if a country is part of a hotspot, its entire microbial count is listed, except in the United States, where the microbial count is listed by state.

Russia (9%), and China (6.7%) were the other predominant Asian countries.

What is clear, and not surprising, is the disproportionate number of organisms that have been isolated in the developed world. The United States, Canada, Europe, and Japan accounted for 73% of the accessions that could be assigned to a geographic location. The continents of Africa and South America and Asian countries not including Japan accounted for only 10.4% of all strains, despite occupying the majority of the world's land mass and accounting for the bulk of the world's population. What is especially daunting is that most tropical regions and areas that are generally thought to be rich in macroflora and -fauna diversity are among the most underrepresented areas in terms of described prokaryotic diversity. This is emphatically illustrated when the number of plant and vertebrate animal species are compared with the number of microbial isolates described from the most biodiverse terrestrial regions on Earth. As shown in Table 3, there is a discrepancy of orders of magnitude between descriptions of microbial species from these environments and descriptions of the macrofauna and –flora species. These trends likely reflect two sampling artifacts in the collection of microbiota. First, most prokaryotic isolates have been described and deposited by researchers likely working close to home at universities or government research agencies in developed countries. Second, the effort required to isolate and describe a new species or strain of microbe takes considerably more financial and technical resources, as well as time, than the effort required to describe a animal or plant species, for which morphological and other visual cues are often the primary requirements for a species description. A third consideration is that microbiologists have focused on isolating organisms with novel physiological properties rather than surveying the natural history of a geographical region.

ENVIRONMENTAL HABITAT DATA

The SOI habitat accounted for 27.3% of all accessions listed and is the largest category by a significant margin (Table 1). Of the soil isolates, 49% were from unknown locations, 16% were from Asia (9% from Japan), 16% were from North America (13.4% from the United States), 10% were from Europe, and the remaining 8.5% came from Africa, Oceania, South America. and the polar regions. On a continental basis, soil organisms comprised the largest category for Africa, Asia, Europe, the polar regions, and unknown geographic locales.

The next largest category was HTP, which accounted for 16% of all accessions. Of these, 33.6% were from unknown locations, 30% were from North America (27% from the United States), 12.3% were from Asia (5.5% from Japan), 11% were from Europe (3% from England), 5% were from Africa, 4% were from Oceania, and 4% were from South America. On a continental basis, HTP organisms comprised the largest category in North America, Oceania, and South America. The SOI and HTP habitats were the only two environmental categories with percentages in the double digits (Table 1).

For the United States as a whole (Fig. 1), the best-represented habitats were HTP (228 entries) and SOI (195 entries). MAC had 101 entries, most of which (65 entries) were from Hawai'i. No other environmental category had 100 or more entries. Of the 300 Japanese entries (Fig. 2), 130 were SOI, while the next largest category was HTP, with 47 accessions. The two most-represented environmental categories for the 189 German accessions were SOI with 48 entries and sewage and manure (SWM) with 30 entries (Fig. 3).

It is interesting to compare the habitat types of isolates that could be assigned to a location in Japan and Germany, the second- and third-largest sources of microbes outside the United States, respectively. Isolates from Japan were overwhelmingly from the soil or were microbes associated with plants, reflecting both the interest in agriculture and a focus on the industrially important actinomycetes. The soil habitat was also dominant in Germany; however, SWM was the second most abundant category, reflecting an interest in agricultural composting and silage, as well as water treatment processes. In Germany, as opposed to Japan, a significant number of isolates were associated with lakes. This reflects the long-standing tradition of limnological research in Germany.

It is also informative to consider habitats that may be underrepresented. First among these are marine habitats, which, combined, accounted for only 10.8% of the total number of accessions; only 0.1% of the accessions were classified as accessions from the open ocean. This is despite the fact that oceans cover two-thirds of the planet and contain an estimated 1×10^{29} prokaryotes (58). Specific reasons for this are hard to identify; however, the difficulty of obtaining samples, especially

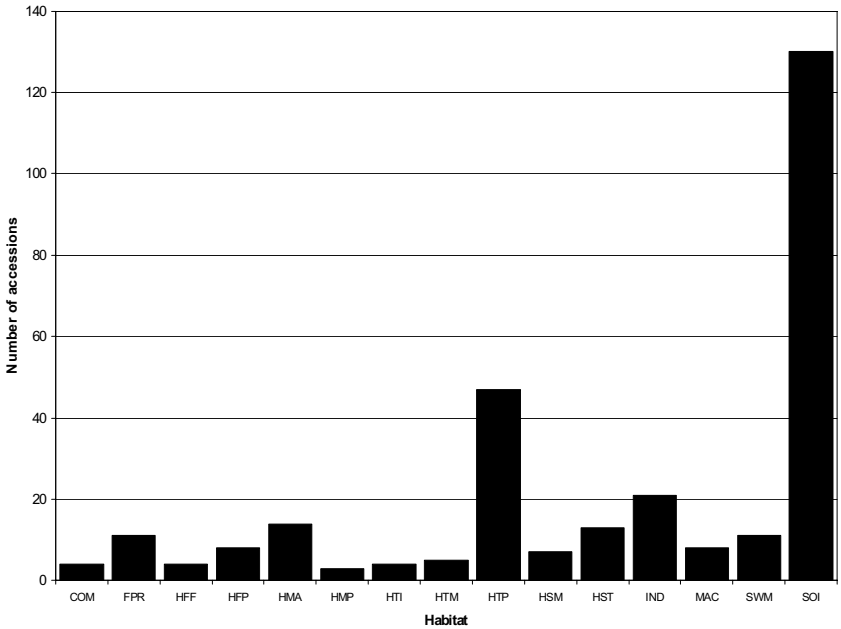

FIG. 2. Japanese environmental accessions by habitat. See Table 1 for an explanation of the codes.

from the open ocean and the depths, and the recalcitrance of many marine microbes to laboratory culture are likely explanations (see below). Another underrepresented habitat was the deep subsurface, which is estimated to contain more microbes than any other habitat on the planet (58). So few isolates at the ATCC fell into this category that it was not even included in our habitat delineation. This undoubtedly reflects the relatively recent discovery of microbes in the deep subsurface within the last 15 to 20 years and the difficulty of sampling them and obtaining novel isolates.

The number of accessions from contaminated sites was also surprisingly low. Although seven of the habitat environmental categories specifically included contaminated sites, these categories accounted for only 1.4% of the accessions (76 entries in all seven categories). Given the amount of research that is done on microbes with the ability to degrade hazardous chemicals and aid in bioremediation, this number of accessions is less than one might expect. While the ATCC has not specifically targeted bioremediation strains for accessioning, there are several possible explanations for the paucity of these

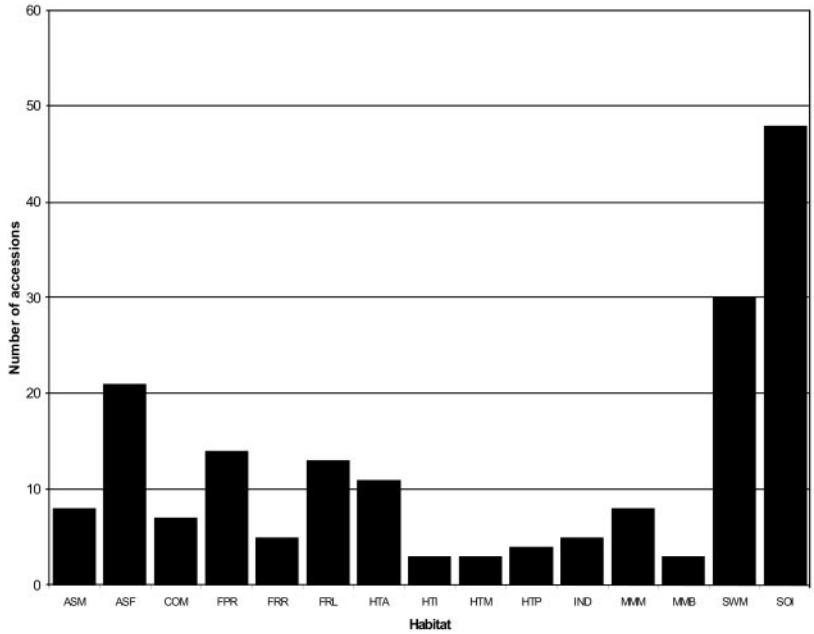

FIG. 3. German environmental accessions by habitat. See Table 1 for an explanation of the codes.

strains. The diversity of biodegradative strains at the genus and species levels is relatively low. The main focus of research is on the function of these organisms rather than their systematics; therefore, while many isolates are obtained, they are only nominally described systematically. It is also possible that due to the biotechnological potential of these organisms they are more often subject to patents and/or intellectual property protection, and such deposits were not included in our analysis.

SPECIFIC HABITATS

Five habitats that were among the habitats with the highest numbers of accessions were chosen for more detailed comparison of the overall diversity of isolates held at the ATCC and for estimates of the diversity in these habitats based on molecular diversity studies. Although these reviews were neither exhaustive nor rigorously quantitative, they did illustrate the strengths and gaps in our ability to culture the important microbes from each of these environments. In this analysis, a phylotype was defined as a group of organisms exhibiting >97% DNA sequence similarity for the 16S rRNA gene, unless otherwise noted.

Soil. An average gram of soil contains on the order of 10^9 prokaryotic cells, and it has been estimated that there could be several thousand bacterial species represented by this number (54). As in other habitats, it is estimated that most soil microbes have not been cultivated using standard techniques. Molecular surveys, largely based on analysis of the 16S rRNA gene, have begun to delineate the extent of microbial diversity in soil and have confirmed that the soil microbial community is very diverse (62). However, there appear to be groups of bacteria that are common to many different soil habitats (4). It is estimated that the most abundant groups of bacteria in soil include, in rough order of relative abundance, the *Acidobacteria*, the α-, β-, and γ-subdivisions of the *Proteobacteria*, and members of the *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Planctomycetes*, and *Verrucomicrobia* (2, 8, 14, 34, 46, 61). The *Acidobacteria*, *Planctomycetes*, and *Verrucomicrobia* are each poorly represented by pure cultures; for example, there are only three described species of *Acidobacteria.*

In some studies workers have specifically compared cultured isolates and 16S rRNA gene clone libraries derived from the same samples. Such comparisons of data from agricultural soils in The Netherlands revealed that the cultured isolates were dominated by members of the *Actinobacteria* and *Firmicutes* (e.g., the genera *Arthrobacter* and *Bacillus*, respectively), while common members of the clone libraries were *Acidobacteria, Verrucomicrobia*, and others (12, 46). When there was overlap between environmental clones and isolates at the genus level (e.g., *Bacillus*), the clones were not closely related to cultured strains. Similar results were found for isolates from arid soils in the southwestern United States (9). The cultured isolates were predominately gram-positive organisms and *Proteobacteria*, whereas environmental phylotypes were dominated by the *Acidobacteria* and included representatives of 10 different phyla.

The dominant genera of ATCC soil accessions are shown in Table 4. Of the 14 most abundant genera from soils, seven belonged to the *Actinobacteria*, including the genus *Streptomyces*. This undoubtedly is a result of both their importance as sources in natural product discovery and their role in shaping the soil environment. Only one genus each represented the different subdivisions of the *Proteobacteria*: *Hyphomicrobium* (α-*Proteobacteria*), *Ralstonia* (β-*Proteobacteria*), and *Pseudomonas* (γ-*Proteobacteria*). However, *Pseudomonas* spp. did account for 6% of the accessions. Members of the phylum *Firmicutes (Bacillus*, *Clostridium*, and *Paenibacillus*) were also in this group, which suggests that these spore formers have probably been overrepresented in the culture collection relative to their importance in the environment. Thus, there was a significant disparity between the diversity captured in the culture collection and what cultivation-independent methods have indicated are the most abundant phylotypes in natural soils.

TABLE 4. Genera represented in the SOI environmental habitat[a]

Genus	No. of entries	% of total
Streptomyces	368	25.2
Bacillus	111	7.6
Pseudomonas	88	6.0
Mycobacterium	38	2.6
Micromonospora	30	2.1
Clostridium	23	1.6
Actinomadura	22	1.5
Actinoplanes	22	1.5
Paenibacillus	21	1.4
Rhodococcus	20	1.4
Arthrobacter	19	1.3
Flexibacter	18	1.2
Hyphomicrobium	17	1.2
Ralstonia	15	1.0

[a] The total number of entries for the SOI habitat was 1,459, and only the genera representing >1% of the total number are shown.

Host-associated terrestrial plant habitat. HTP was the second largest category in the study, and this undoubtedly reflects the impact of agriculturally based microbial research on accessioning at the ATCC. The interaction between plants and the microbial community is complex. Above ground, the leaf surfaces, or phyllosphere, of plants harbor an abundant and diverse community of microbes adapted to this environment (31). Below ground, plant roots release a wide range of substances that serve as nutrients, stimulants, and inhibitors which affect microbial growth in the rhizosphere (5) and select for quite a different microbial community. In addition, plant stems, flowers, and seeds may also harbor unique communities (40).

The two primary areas of research for HTP microbes have to do with their detrimental role as plant pathogens and the beneficial role that they play in plant nutrition, principally as nitrogen-fixing symbionts. These focal areas were reflected in the most abundant HTP organisms in the ATCC collection, as shown in Table 5. The two most abundant groups, *Xanthomonas* and *Pseudomonas*, both contain a number of plant pathogens, although some *Pseudomonas* spp. may have beneficial effects as well. On the other hand, the symbiotic members of the *Rhizobiales* (*Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, and *Sinorhizobium*) all play a vital role in providing fixed nitrogen to leguminous plants (33).

Relative to the other habitats considered here, there have been few culture-independent assessments of HTP communities. In one such study of the phyllosphere the workers investigated a limited number of 16S rRNA gene clones and found

TABLE 5. Genera represented in the HTP environmental habitat[a]

Genus	No. of entries	% of total
Xanthomonas	170	20.0
Pseudomonas	117	13.7
Pectobacterium	44	5.2
Erwinia	46	5.1
Rhizobium	38	4.5
Pantoea	29	3.4
Spiroplasma	29	3.4
Streptomyces	24	2.8
Burkholderia	23	2.7
Clavibacter	20	2.4
Bacillus	19	2.2
Bradyrhizobium	19	2.2
Xylella	19	2.2
Brenneria	18	2.1
Sinorhizobium	18	2.1
Azospirillum	15	1.8
Curtobacterium	12	1.4
Clostridium	9	1.1
Gluconacetobacter	9	1.1
Mesorhizobium	9	1.1

[a] The total number of entries for the HTP habitat was 852, and only the genera representing >1% of the total number are shown.

TABLE 6. Genera represented in the HTM environmental habitat[a]

Genus	No. of entries	% of total
Escherichia	33	11.5
Streptococcus	24	8.4
Clostridium	14	4.9
Staphylococcus	11	3.9
Dermatophilus	8	2.8
Lactobacillus	8	2.8
Macrococcus	8	2.8
Corynebacterium	6	2.1
Fibrobacter	6	2.1
Flexispira	6	2.1
Treponema	6	2.1
Enterococcus	5	1.8
Eubacterium	5	1.8
Acidaminococcus	4	1.4
Brachyspira	4	1.4
Hespellia	4	1.4
Listeria	4	1.4
Veillonella	4	1.4

[a] The total number of entries for the HTM habitat was 286, and only the genera representing >1% of the total number are shown.

that there appeared to be significant differences between the environmental clones and the microbes that are typically cultured from leaf surfaces; for example, this study found several clones related to the δ-*Proteobacteria* (59). In the rhizosphere the story was more complex because it is not easy to differentiate between the bulk soil community and the true rhizosphere community (i.e., the microbes that are present only due to their association with plant roots) (27). Molecular studies have indicated that there are differences between soil and rhizosphere communities (29). Based on culture-independent surveys of soils, many of which have a rhizosphere component (see above), it is certainly reasonable to assume that many rhizosphere microbes have not been represented in culture.

Host-associated terrestrial mammals (HTM). It is now well recognized that the bulk of cell types that make up a mammal are prokaryotic and not animal cells (63). An abundant and defined microbial flora occupies the skin and the gastrointestinal tract of all mammals. These associations have been the subject of many studies, although the bulk of the studies have been on humans and the organisms thus obtained were not included in our analysis. We could find little information on molecular surveys of the epidermis of nonhuman mammals, and so we focused on those studies investigating the gastrointestinal tract, where microbial communities play a crucial role both in nutrition and in controlling susceptibility to disease (63).

In a landmark study workers recently investigated the porcine gastrointestinal tract. Leser et al. isolated 4,270 16S rRNA gene clones from the ileum, cecum, or colon of several different pigs (30). They identified 375 different phylotypes among these clones, 83% of which were unique. Thirteen major phylogenetic lineages were represented; however, the large majority of clones belonged to a few lineages, chiefly the *Firmicutes* (81%), and the *Bacteroidetes* (11.2%). Among the *Firmicutes*, the clostridia and lactic acid bacteria were especially common, and these organisms are among the most commonly cultured microbes from the porcine gut as well.

A molecular clone study in which bovine rumen fluid and rumen solids were examined revealed that 52.4% and 71.4% of the clones, respectively, belonged to the *Firmicutes*, while *Bacteroidetes* accounted for 38.1% and 26.2% of different rumen populations (53). The *Spirochaetes* accounted for 2 to 3% of the rumen population. Similarly, a study of 16S rRNA gene clones from the nonruminant equine large intestine showed that out of 272 clones, 168 could be assigned to unique phylotypes; of these, 72% fell in the *Firmicutes*, while 20% were members of the *Bacteroidetes* (7). The *Spirochaetes* and *Verrucomicrobia* each accounted for 3% of the clone libraries. Results similar to these were also obtained in a study of African herbivores (36)

What is clear from these studies on the gastrointestinal tracts of mammals is that the numbers and macrodiversity of phylotypes present were significantly less than the numbers and macrodiversity of non-host-associated environments; nevertheless, the microdiversity was still high. Comparison with the best-represented HTM genera in the ATCC collection (Table 6) confirmed that members of the *Firmicutes* were abundant, accounting for 11 of the 18 genera listed. *Clostridium* spp. represented the third most abundant genus in the collection; members of this genus and its close relatives accounted for the most abundant group of phylotypes in the majority of the molecular studies cited above. *Escherichia*, primarily *Escherichia coli* (γ-*Proteobacteria*), was the most abundant genus in the collection, but it was almost certainly oversampled relative to its true abundance, probably due to its pervasive use as a model organism. What is perhaps surprising is that no members of the *Bacteroidetes* were included Table 6, although members of this phylum accounted for approximately one-third of the phylotypes found in molecular analyses. Compared to the other habitats examined here, the HTM isolates showed the best concordance with findings of molecular surveys, although there was still little overlap between the specific phylotypes found in molecular surveys and those found in pure culture.

Freshwater aquatic diversity. Freshwater lakes span a wide variety of habitat types, ranging in size from shallow ponds to large bodies of water like the Laurentian Great Lakes and nutritionally from ultraoligotrophic lakes of polar zones to eutrophic lakes in agricultural and urban regions heavily impacted by fertilizer runoff and other pollutants. Despite this inherent diversity of habitat types, a picture of lake communities as communities having a cosmopolitan makeup is beginning to emerge based on molecular analyses of different lakes from around the world. In 2002, Zwart et al. compiled and analyzed a database of 689 bacterial 16S rRNA gene sequences that came from the water columns of lakes or rivers (64). Their results suggested that the groups common to freshwater ecosystems include the α-, β-, and γ-*Proteobacteria*, the *Bacteroidetes*, the *Cyanobacteria*, the *Actinobacteria*, the *Verrucomicrobia*, and the green sulfur bacteria (*Chlorobi*). With the exception of the *Cyanobacteria* and *Chlorobi*, these phyla are also common members of soil and/or marine habitats; however, the specific clades found in freshwater tended to be unique compared to those associated with soil or marine habitats (57). The majority of clades associated with freshwater did not have close known relatives in culture. A number of molecular studies investigating individual lakes or groups of lakes have shown that the overall bacterial diversity is substantial, although the majority of organisms tend to fall within the clades described above (15, 20, 48, 60, 65).

Several studies of lakes in which the workers compared cultured isolates with molecular surveys have been done (25, 39) One such comparison was done for an oligotrophic lake in Antarctica (39). The cultured isolates were predominately fluorescent and nonfluorescent *Pseudomonas* species, as well as members of a number of other taxa, including *Vibrio*, *Aeromonas*, *Alcaligenes*, *Actinobacteria*, and *Micrococcus*. Molecular analysis based on the 16S rRNA gene revealed some overlap with cultured strains at the phylum or family level but much less correspondence at the genus or species level. Molecular analysis also revealed the presence of spirochetes and *Verrucomicrobia* that were not among the cultured isolates.

Table 7 shows some of the most abundant bacteria in the ATCC collection that were isolated from freshwater lakes (FRL). The most abundant genera were *Synechococcus*, a member of the *Cyanobacteria*, followed by *Aquaspirillum*, *Caulobacter*, and *Pseudomonas*, which are members of the β-, α-, and γ-*Proteobacteria*, respectively. Another phylum represented with some abundance was *Bacteroidetes* (*Flavobacterium* and *Flexibacter*). *Planctomycetes* and *Verrucomicrobia* were represented by the genera *Pirellula* and *Prosthecobacter*, respectively. The dominant phylum from FRL clone libraries that was not represented with any frequency in the ATCC holdings was the *Actinobacteria*. Thus, there does appear to be some concordance between the phyla of isolates and the phyla shown to be abundant in FRL by culture-independent methods. As is the case with other environments, however, at the species or phylotype level there is little specific overlap between organisms suggested to be important by 16S rRNA gene clonal analysis and organisms that have been isolated and deposited in the collection.

TABLE 7. Genera represented in the FRL environmental habitat[a]

Genus	No. of entries	% of total
Synechococcus	15	7.5
Aquaspirillum	13	6.5
Caulobacter	11	5.5
Pseudomonas	9	4.5
Prosthecomicrobium	7	3.5
Aquamonas	5	2.5
Brevundimonas	5	2.5
Leptolyngbya	5	2.5
Xanthobacter	5	2.5
Flavobacterium	4	2.0
Lysobacter	4	2.0
Nostoc	4	2.0
Rhodobacter	4	2.0
Blastobacter	3	1.5
Cellulomonas	3	1.5
Chroococcidiopsis	3	1.5
Flexibacter	3	1.5
Herpetosiphon	3	1.5
Methanobacterium	3	1.5
Prosthecobacter	3	1.5
Sphaerotilus	3	1.5

[a] The total number of entries for the FRL habitat was 201, and only the genera representing >1% of the total are shown.

Coastal marine habitat. An analysis by Hagström et al. of accessions of small-subunit rRNA genes from all marine microbes listed in the GenBank database revealed a total of 1,117 unique ribotypes (17). The number of newly described ribotypes had remained relatively constant for several years, causing the authors to speculate that species richness in the ocean is relatively low. The most extensive molecular analysis of any single microbial community was recently completed for the Sargasso Sea (56). This study, based on shotgun sequencing and analysis of multiple environmental genomes, found at least 1,800 genomic species in 1,500 liters of open ocean water. Of these, 148 (8%) were new phylotypes or putatively new species, again suggesting that the discovery rates for new species in the ocean may be modest. However, these authors point out, as have others (13), that more extensive genomic analysis beyond the 16S rRNA gene reveals that genotypic diversity, and by extension phenotypic diversity, may be much more significant among the bacterioplankton than 16S rRNA gene phylogenies suggest.

In general, molecular analyses of coastal waters have shown that the α- and γ-*Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* are the most dominant groups (1, 42, 50–52). Two of the prevalent groups within the α-*Proteobacteria* are the SAR11 cluster and the *Roseobacter* clade. Commonly found members of the γ-*Proteobacteria* include *Alteromonas*, *Pseudoalteromonas*, and the SAR86 cluster. Members of the phylum *Bacteroidetes* often account for between 10% and 20% of coastal marine clone libraries as determined by fluorescent in situ hybridization studies (28). A recent study of this phylum from coastal waters of the United Kingdom found little overlap in dominant phylotypes in cultivated and noncultivated populations (37). Other cosmopolitan bacterioplankton phyla include members of the *Actinobacteria* and the *Verrucomicrobia*; these often account for several percent of the clone libraries. Other comparative studies of marine habitats have revealed little overlap between cultured isolates and environmental clones (52, 11). One exception to this was an analysis of a nutrient-rich Chinese estuary; there, better concordance was found

TABLE 8. Genera represented in the MAC environmental habitat[a]

Genus	No. of entries	% of total
Vibrio	44	19.0
Pseudoalteromonas	29	12.5
Halomonas	14	6.0
Photobacterium	10	4.3
Unidentified	9	3.9
Alteromonas	7	3.0
Listonella	7	3.0
Marinomonas	7	3.0
Shewanella	6	2.6
Marinobacterium	5	2.2
Oceanospirillum	5	2.2
Bacteriovorax	3	1.3
Cobetia	3	1.3
Hyphomonas	3	1.3
Marinobacter	3	1.3
Oceanimonas	3	1.3
Oceanobacter	3	1.3

[a] The total number of entries for the MAC habitat was 232, and only the genera representing >1% of the total are shown.

among clones and isolates in the genera *Alteromonas* and *Roseobacter* (45).

The marine coastal environment is another case where there is a significant discrepancy between the phylogenetic types that are predominant in the culture collection and the phylogenetic types that are found in molecular surveys. Table 8 lists the 16 most abundant genera of bacteria isolated from marine coastal waters that were in the ATCC; 14 of them belong to the γ-*Proteobacteria*. The isolates from the coastal marine environment are dominated by *Vibrio* and *Pseudoaltermonas* spp. Based on cultivation studies, *Vibrio* has long been thought to be a typical marine microbe; however, molecular surveys of coastal marine diversity have found that *Vibrio* is quite rare (see references above). Members of the genus *Pseudoalteromonas* were well represented in the culture collection and, as mentioned above, have also been found commonly in molecular surveys of coastal waters. Despite this overlap at the genus level, most clones identified through molecular surveys do not match cultured representatives of *Pseudoalteromonas*.

New culture techniques hold promise. The case studies described above paint a bleak picture of the divide between the organisms that are well represented in culture collections and the dominant members of their habitats. Fortunately, in the last few years more effort has been devoted to cultivation approaches that better mimic in situ conditions and are aimed at capturing the underrepresented groups. A few examples are given below for soil, freshwater, and marine habitats.

Janssen and colleagues have isolated novel members of the *Acidobacteria* from soil using a nutrient-poor plating medium with a polymeric substrate, xylan. In addition, they described isolates that belonged to novel groups in the α- and γ-*Proteobacteria*, as well as *Actinobacteria* that had previously been identified through clone libraries but had not been cultured (24, 43). In another recent study the workers used similar low-nutrient strategies to isolate previously uncultured members of the *Acidobacteria* and the *Verrucomicrobia* from soils in Michigan (49).

The work of Hahn and coworkers has led to a breakthrough in cultivating members of the *Actinobacteria* that have been found in a number of clone libraries from lake water. By using a dilution plating technique, these researchers isolated two new clades of *Actinobacteria* that appeared to be numerically abundant but had not been cultured previously (19). Using similar techniques, they were able to describe *Polynucleobacter*, a new cosmopolitan genus belonging to the β-*Proteobacteria* (18) from several lakes around the world. Furthermore, their isolates closely matched a group of β-*Proteobacteria* that was originally known only through analysis of clone libraries from lakes. Similarly, Bruns et al. used a variety of signal compounds to increase the culturability of bacterioplankton from a eutrophic lake in Germany (3). The most effective compound was cAMP, and they were able to cultivate previously uncultured β-*Proteobacteria* and members of the *Actinomycetales*.

Recent improvements in culture technique have also begun to open the "black box" of unculturable marine species. An example is the Sar11 cluster, which has long resisted laboratory culture. By using very dilute seawater amended with micromolar amounts or less of nitrogen, phosphorus, and organic substrates, Rappe et al. were able to cultivate members of the ubiquitous Sar11 clade (41). The *Roseobacter* clade is another underrepresented group in terms of the numbers of pure cultures. These organisms are amenable to cultivation using more standard techniques (16, 35), yet they have been discovered only relatively recently and are not well represented in culture collections. This reveals another service of molecule-based diversity surveys, recognizing important groups of organisms which are amenable to laboratory culture but are simply undersampled.

Clearly, culture collections must have an important role in the preservation and maintenance of these novel organisms, as well as authenticating them. Toward this end, the International Journal of Systematic and Evolutionary Microbiology has recently required that all validly named type strains now be deposited in two recognized culture collections in two different countries (38). In addition to serving as repositories for microbes, culture collections can also act as more comprehensive bioresource centers by being clearinghouses for information about the provenance and characteristics of the microbes that they acquire. While this information takes time to accumulate, the longevity and mandate of microbial culture collections should ensure that, once collected, it will be available for microbiologists for generations to come.

CONCLUSIONS

Analysis of the geographic locations and habitat types of environmental isolates at the ATCC showed that there were significant disparities between both locations and habitats that were represented in the culture collection. While it is anecdotally assumed that prokaryotic species are much better sampled in the major scientific countries of the developed world, this work underscores, with numbers, the degree to which the anecdotes are true. From an ecological perspective, the paucity of isolates and data about microbial abundance and diversity from the tropics is of concern. It is a well-established tenet of animal and plant ecology that species diversity is highest in the tropics and decreases toward the poles (47). The state of our knowledge is such that we cannot begin to answer whether patterns of microbial diversity follow the same trends. A com-

parison between molecular phylogenetic surveys and ATCC holdings for select habitats that were relatively well represented by isolates showed that although there is general concordance at the family level and often at the genus level, some phylum-level groups are significantly underrepresented in the collection. It is apparent that the phyla *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes* are especially underrepresented in cultured isolates. Recent breakthroughs in culturing the "unculturable majority" of prokaryotes offer hope for redressing some of these disparities. Given the sheer abundance and diversity of prokaryotes and the significant effort involved in isolating, characterizing, and maintaining them, intelligent strategies need to be developed if we are to capture a reasonable, viable subset of the Earth's prokaryotic diversity.

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